

**Image Analysis in the Evaluation of the Physical and Physiological Quality of Jiló
(*Solanum gilo*) Seeds During Development**

ABSTRACT

Currently, the X-ray image analysis of seeds has proved effective for the evaluation of the physical and physiological quality of the seeds, besides being a non-destructive method. The objective of this work was to evaluate the internal free area of jiló seeds (*Solanum gilo*) through the analysis of radiographic images and to relate the results with the germination of seeds in different stages of maturation. The seeds were harvested at 35, 40, 45 days after anthesis and 45 days after anthesis with 7 days at rest (45 DAAr). Seeds with free internal area $\leq 10\%$ produced normal seedlings whereas seeds with an average free area above 10% produced abnormal or non-germinated seedlings. Seeds of fruits harvested at 45 DAA and seven days at rest showed a smaller internal free area and a formation of 100% of normal seedlings. The analysis of the radiographic images of jiló seeds allows the measurement of the free internal areas, as well as the determination of the relation between these and the germination.

Keywords: Germination; ImageJ®; Physiological maturity; Seed development; X-ray.

1. INTRODUCTION

Originating from India and introduced in Brazil by slaves, jiló (*Solanum gilo* Raddi) is a tropical heat-demanding vegetable. It belongs to the Solanaceae family and its fruits are of light green or dark green color when immature, becoming orange reddish when ripe [1]. Jiló is a vegetable of great acceptance in the market, mainly in the southeast region, but, in general, it is still a species that require further studies, mainly in the area of seeds [2].

Several authors describe the importance of high quality of this seed as determinant in the production of the plant in the field. The degree of maturation of the seeds influences its quality, and the immature seeds have low vigor and germinative Power [3,4,5]. In the present study, the analysis of seed and seedling images has been shown to be efficient for the evaluation of the physiological and physical components of seeds.

Seed analysis is considered a constantly evolving dynamic activity, characterized by continuous improvements in process development and standardization. For Guedes et al. [6], the standardization

36 of these methods should be constantly re-evaluated through the application of reference tests,
37 alternative tests and the determination of new methodologies.

38 Studies on seed analysis using radiographic images are a relatively recent alternative to classify the
39 various aspects of seeds, such as their internal morphology, mechanical damage, insect damage,
40 among others. In this sense, the capture and processing of the radiographed image has allowed the
41 establishment of relations between integrity, morphology and determination of the physiological
42 potential of the seeds [7].

43 Thus, one of the basic requirements for the identification of problems associated with the
44 physiological potential of seeds is the investigation of their internal morphology. Studies aimed at
45 evaluating the internal morphology of seeds have been performed using the image analysis technique.
46 Among the methods used for this purpose, the X - ray test stands out [8].

47 In addition to all of these possibilities of use, the X - ray test has been successfully performed to
48 relate the internal morphology of the seed with the germination or morphology of tomato seedlings
49 [9], sweet pepper [10,11], eggplant [5], melon, pumpkin and watermelon [8], *Acca sellowiana* [12],
50 pumpkin [13].

51 However, in most cases, this classification is performed visually, and parameters that are more
52 precise are needed to develop more consistent models of evaluation to define categories of extension
53 of embryonic development or free space within the seeds [7].

54 Thus, the objective of this research was to relate the internal morphology of jiló seeds (*Solanum*
55 *gilo*) at different stages of maturation with germination through radiographic images.

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57 2. MATERIALS AND METHODS

58 2.1. Seed Collection Location

59 Research was carried out at the Center for the Development and Technology Transfer of UFPA at
60 the Hortiagro Sementes Company, Municipality of Ijaci, MG, and in the Central Seed Laboratory of
61 the Department of Agriculture of the Federal University of Lavras.

62 At the first stage of the study in a greenhouse, jiló seedlings were formed for the installation of the
63 experiment and seed production. The seeds were sown on commercial substrate in 128-cell trays. The
64 seedlings were transplanted into a greenhouse when they showed four definite leaves.

65 During the flowering season, emasculation and manual pollination were carried out to produce
66 hybrids. The flowers were labeled daily on the day of the anthesis until the necessary number of fruits
67 to perform the analysis of each treatment. Six commercial genetic materials, JIL 001, JIL 006, JIL
68 white and cultivar JIL - 005 (pollen donor) and two hybrids H1 and H2, from the crosses between JIL
69 - 005 (♂) X JIL - 001 (♀) and JIL-005 (♂) X JIL-006 (♀) were used. The crosses were conducted
70 manually, after the emasculation of the female parentals. Fruit harvesting was performed at 35, 40, 45
71 days after anthesis (DAA) of the first flowering. Half of the number of fruits harvested at 45 DAA

72 was put to rest for seven days (45 DAAR) in a cool and ventilated place, totaling 24 treatments (six
73 genetic materials and four stages of fruit development).

74 The experiment was installed in a randomized block design (RBD) with 24 treatments and three
75 replications, where each plot was composed of 1 row of 6 meters long with 10 plants, spaced 1 meter
76 between rows and 0.60 m between plants.

78 2.2. Laboratory Experiments

79 After the seeds were extracted from the fruits, the seeds were dried in an air circulation oven at 35
80 °C until they obtained 8% water content. Then the imaging tests and physiological analysis were
81 performed.

82 For the radiographic analysis, four replicates of 50 seeds of each treatment were used, placed on a
83 transparent adhesive tape (double face) and fixed in transparent plastic slide; the seeds of each
84 treatment were numbered according to the position occupied in the slide, so that they could be
85 identified in subsequent determinations.

86 The plastic slide was placed inside the X-ray digital equipment, Faxitron® brand, model MX-20
87 DC-12, and subject to radiation for 12 seconds at 26 kV. Then, the generated images were saved to
88 the computer's hard disk for analysis. The seeds were removed from the transparent slide and
89 transferred to acrylic boxes, gerbox type, being arranged in the same order as they were in the X - ray
90 images. After this procedure, they were conducted to the germination test.

91 The germination test was performed on two sheets of paper moistened with distilled water 2,5
92 times the paper mass. The seeds distributed in gerbils in the same positions where they were in
93 radiographic images and maintained in BOD chambers under alternating temperature and light
94 regime, being 20 °C / 16 hours in the dark and 30 °C / 8 hours in the presence of light. The count was
95 performed 14 days after sowing [14]. Normal (PN), abnormal (PA) and dead seed (SM) seedlings
96 were photographed using a Canon SX50® digital still camera. Images were saved to the computer's
97 hard drive for further analysis.

99 2.3. Measurement of the free internal area of jiló seeds

100 The images of the radiographed seeds were saved in the JPEG format and analyzed with the
101 ImageJ® software [15], adapting the analysis methodology previously used in other studies to measure
102 leaf area and internal area of seeds, Silva et al. [12], according to example shown in Fig. 1.

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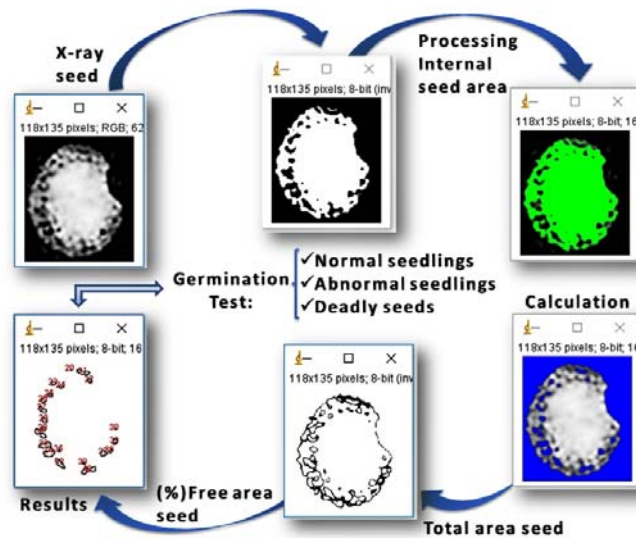


Fig. 1. General representation of jiló seed image analysis, from radiographic image to the attainment of free internal area and germination tests.

The steps of the analysis in the ImageJ® program were taken as follows: image opening, and its conversion to grayscale type in 8 bits. Then, the area of interest for image analysis and calibration was selected. In this study, the value in squared millimeters (mm^2) was considered for each image as a reference, which was 231 X 210 mm. For the choice of parameters to be measured, perimeter, standard deviation and fraction area were chosen as object area, which represents the free internal areas of the seeds. The color adjustment was performed to separate the areas of interest from the other constituents of the image. And finally, parameters were measured and results obtained. All procedures were performed manually for each seed.

The free area of the internal seed cavity in each treatment ($n = 200$) was calculated by means of the ImageJ® software in four replicates of 50 seeds. After the attainment of radiographic images, 3D images and their histograms of jiló seeds harvested at the different stages of maturation were processed with ImageJ® software.

2.4. Statistical analysis

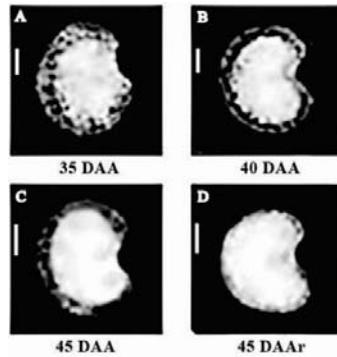
For the analysis of variance in the germination test and in the image analysis of the seeds, the statistical program Sisvar was used [16]. For the comparison between the means, the Scott-Knott [17] test was employed, at 5% probability.

3. RESULTS AND DISCUSSION

3.1. Radiographic images and free internal area of seeds

128 Through the analysis of the radiographic images, it was possible to see the internal area of the jiló
129 (*Solanum gilo*) seeds and to identify different percentages of free internal areas in different stages of
130 maturation. The free areas inside the seeds were visualized in all genetic materials.

131 The overall mean of the free internal areas of all treatments, with 25, 18, 6 and 4%, for seeds
132 harvested at 35, 40, 45 DAA and 45 DAAr (seeds harvested at 45 DAA and left at rest for seven
133 days), respectively, are shown in Fig. 2.



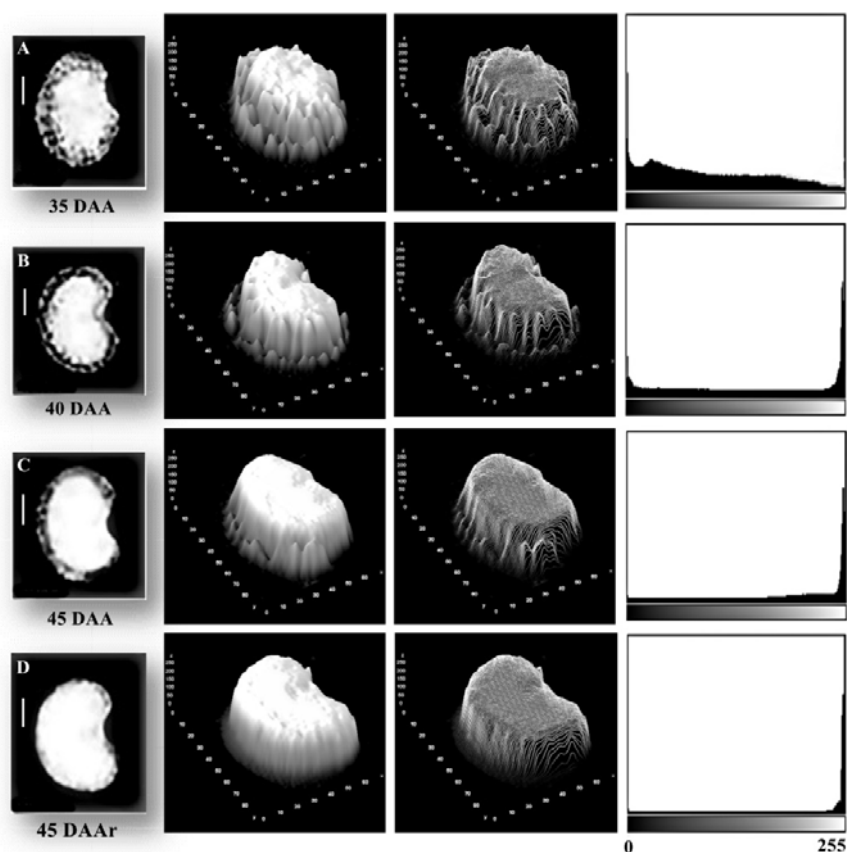
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135 **Fig. 2. Sequence of radiographic images of jiló seeds during the maturation process, A (35**
136 **DAA), B (40 DAA), C (45 DAA), and D (45 DAAr). Bars are 5 mm.**

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138 The shade found in radiographic imaging is defined according to level of radiation absorption in
139 different regions of the seed, which is determined by the thickness, density and composition of the
140 seed tissues [18, 19]. Thus, seeds that do not have embryonic tissue, caused by the absence of
141 resistance to the passage of X - rays, provide dark images. After the attainment of the radiographic
142 images, 3D images and their histograms on jiló seeds harvested at different maturation stages were
143 visualized with the aid of ImageJ[®] software (Fig. 3).

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146 **Fig. 3. Radiographic images of jiló seeds at different maturation stages (35, 40, 45 DAA and 45**
 147 **DAAR), and their respective 3D images and their histograms in pixels (0 to 255). Bars are 5 mm.**

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149 According to (Fig. 3), in the initial stages of seed maturation (35 and 40 DAA), the radiographic
 150 images (A and B) present larger dark areas, with a larger number of cavities in the 3D images, and the
 151 peaks in histograms occur on the left side, with darker shades (larger free internal areas).

152 In seeds harvested at 45 DAA and 45 DAAR, the radiographic images (C and D) are clearer and
 153 have smaller free internal areas. From the 3D images in these stages, the number of cavities in the
 154 seeds (seed filling) decreased, and peaks in histograms moved to the right (seeds with smaller free
 155 areas).

156 Additional research showed the efficiency of the X - ray image analysis for the evaluation of the
 157 internal area of canafistula seeds [20], sweet pepper [10], eggplant [5], melon, pumpkin, and
 158 watermelon [8] and pumpkin [13].

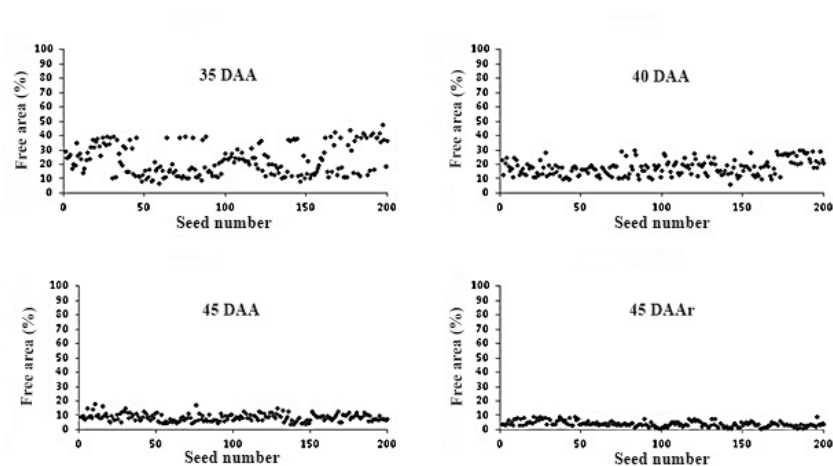
159 The analysis of the radiographic images with the help of the ImageJ[®] software enabled the
 160 measurement of the free internal areas of the seeds and, thus, allowing the evaluation of the relation
 161 between the free internal area of the seeds and the germination.

162 This result is important because the software reported in the literature for this type of analysis are
 163 Tomato Analyzer[®] [7] and Image ProPlus[®] [11,5], whereas in the modern literature review, no image

164 analysis study was performed on jiló (*Solanum gilo*) seeds using ImageJ[®] software, but in *Acca*
165 *sellowiana* seeds [12].

166 In light of the foregoing, the software can be an alternative for research on different seed species in
167 this area of study, since methods that use capture and image processing at high-speed are the most
168 advanced techniques that can provide a high level of efficiency for analysis of seed quality [21].

169 Based on the results obtained in the image analysis with the ImageJ[®] software, there was a
170 progressive decrease of the free internal area of seeds in all genetic material with the process of seed
171 maturation. General average distribution of the free area within each seed (n = 200) of the genetic
172 material JIL white, JIL 001, JIL 005, JIL 006, H1 and H2 are shown in Fig. 4.



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174 **Fig. 4. Average free internal area (%) of each jiló seed (*Solanum gilo*) in a sample of 200 seeds,**
175 **representing all the genetic material at 35, 40, 45 DAA and 45 DAAR.**

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177 According to Carvalho and Nakagawa [25], the more distant from the anthesis the more fruits are
178 harvested, the heavier the seeds will be. Miranda et al. [22], studying the maturation of eggplant fruits,
179 concluded that there was no difference in the dry weight gain between seeds harvested at 50 DAA in
180 relation to seeds harvested at 60 DAA. Oliveira et al. [23], working with sweet pepper seeds,
181 concluded that at 55 DAA, the seeds had reached physiological maturity, and this was the point where
182 the seeds reached a higher dry mass, coinciding with the best harvesting point.

183 Thus, with the advancement at the stage of maturation of jiló seeds, there was an increase of dry
184 mass and consequent decrease of free internal area. The evaluation of the internal seed morphology is
185 essential both for the characterization of poorly studied species and for the improvement of the quality
186 of seed lots, with regards to their physical and physiological attributes, since information on the
187 existence of defective and empty seeds is desirable because they may influence germination results
188 [24].

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192 **3.2. Germination test**

193 It was possible to relate the results of the germination test with the percentage of free internal area
194 of the seeds, normal and abnormal seedlings and dead seeds. In seeds of all genetic material harvested
195 at 35 DAA, germination did not occur with 100% of dead seeds and with a mean free area within the
196 seed of 25%.

197 At 40 DAA in seeds of the genetic material JIL white, JIL 001, JIL 005 and JIL 006, there was a
198 mean percentage of free area inside the seed of 18%, and average germination percentage of 10%,
199 without the formation of normal seedlings, with an average of 21% of abnormal seedlings and 75% of
200 dead seeds. Whereas for the hybrids H1 and H2, at 40 DAA, germination of 35 and 25% occurred,
201 respectively, however, with a low percentage of normal (15 and 5%) and abnormal (10 and 20%)
202 seedlings.

203 At 45 days after anthesis (45 DAA), there was seed germination percentage of white JIL, JIL 001,
204 JIL 005 and JIL 006, H1 and H2 of 80, 86, 55, 84, 98 and 96%, respectively with the same percentage
205 of normal seedlings, no formation of abnormal seedlings and 20, 14, 45, 16, 2 and 4% of dead seeds.
206 Whereas seeds originating from fruits harvested at 45 DAAR showed a mean percentage of free
207 internal area of 4%, and 100% of germination of normal seedlings, except for the JIL white cultivar,
208 with 95% germination.

209 Seeds from fruits harvested at 45 DAAR of cultivars JIL white, JIL 001, JIL 005 and JIL 006 were
210 statistically superior to seeds harvested at (35, 40 and 45 DAA). In these seeds, higher percentages of
211 germination and the lowest percentages of free internal area were found, except in seeds of hybrids
212 H1 and H2, in which there were no statistical differences of the percentage of germination at 45 DAA
213 and 45 DAAR.

214 There was a direct relationship between the germination and the free area inside the seeds, since
215 there was a greater germination in seeds with a smaller free internal area. The lowest values of free
216 internal area and higher germination were found in seeds of hybrids H1 and H2 from the 45 DAA
217 stage (Table 1).

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Table 1. Free internal area in the jiló seed (FA (%)), germination (G), normal seedlings percentage (NS), abnormal seedlings (AS), and dead seeds (DS).

JIL White					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	32 d	0 c	0	0	100
40 DAA	24 c	0 c	0	25	75
45 DAA	10 b	80 b	80	0	20
45 DAAr	5 a	95 a	95	0	5
CV ¹	8.1	9.4			
JIL 001					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	22 d	0 d	0	0	100
40 DAA	17 c	10 c	0	20	80
45 DAA	10 b	86 b	86	0	14
45 DAAr	5 a	100 a	100	0	0
CV ¹	6.7	5.7			
JIL 005					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	26 d	0 c	0	0	100
40 DAA	21 c	0 c	0	25	75
45 DAA	10 b	55 b	55	0	45
45 DAAr	5 a	100 a	100	0	0
CV ¹	13.3	12.7			
JIL 006					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	25 d	0 d	0	0	100
40 DAA	18 c	9 c	0	17	83
45 DAA	9 b	84 b	84	0	16
45 DAAr	4 a	100 a	100	0	0
CV ¹	7.5	5.3			
H1					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	22 c	0 c	0	0	100
40 DAA	13 b	35 b	15	10	75
45 DAA	4 a	98 a	98	0	2
45 DAAr	3 a	100 a	100	0	0
CV ¹	14.7	4.6			
H2					
Stage	FA (%)	G (%)	NS (%)	AS (%)	DS (%)
35 DAA	23 d	0 d	0	0	100
40 DAA	15 c	25 c	5	20	75
45 DAA	5 b	96 a	96	0	4
45 DAAr	3a	100 a	100	0	0
CV ¹	6.6	7.2			

231 Means followed by the same lower case letters in the columns do not differ by the Scott-Knott test at
232 5% probability. ¹Coefficient of variation.

233 Similar results related to the reduction of free area and the increase in germination originating from
234 fruits harvested at different maturation stages in sweet pepper species were found by Oliveira et al.
235 [23], Carvalho and Nakagawa [25], Nakada et al. [26], and Santos et al. [27].

236 In general, for all genetic materials studied (JIL white, JIL 001, JIL 005, JIL 006, H1 and H2),
237 fruits harvested at 35 DAA with mean free area $\geq 22\%$ did not germinate and 100 % of the presented
238 dead seeds.

239 Seeds from fruits harvested at 40 DAA with an average free area of $\geq 15\%$ produced only
240 abnormal seedlings for JIL white, JIL 001, JIL 005, JIL 006 and with a mean percentage of 80% of
241 dead seeds. Where as for the seeds of hybrids H1 and H2 germination at 40 DAA, however very low
242 and with the presence of abnormal seedlings.

243 Seeds from fruits harvested at 45 DAA, with an average free area of $\leq 10\%$ produced on average
244 84% of normal seedlings, and there was no formation of abnormal seedlings. Where as seeds of fruits
245 harvested at 45DAA and resting for seven days showed 100% normal seedlings and an average free
246 area of 4%.

247 It stands out that the presence of abnormal seedlings for genetic materials, JIL white, JIL 001, JIL
248 005, JIL 006, at 35 and 40 DAA, may be directly related to seed immaturity and the high percentage
249 of free area within these. This can be found in the evaluation of X - ray images, together with the
250 ImageJ[®] program, and also associated to the results of physiological quality.

251 In a similar study, in sweet pepper seeds, when the free space between the embryo and the
252 endosperm was superior to 2.7%, that is, seeds with endosperm area and embryo inferior to 97.3%,
253 there was a reduction of germination with the increase in the percentage of abnormal or non-
254 germinated seedlings [11].

255 In several researches, it has been reported that partially formed seeds were not able to provide
256 normal seedlings in the germination test, such as *Cecropia pachystachya* Trec. [28], Peruvian pepper
257 [29], sweet pepper [11], eggplant [5], watermelon [8], pumpkin [13]. However, for many species of
258 fleshy fruits, fruit harvesting followed by a resting period for 7 or 10 days positively interferes with
259 seed quality [30,31].

260 The analysis of radiographic images of jiló seeds (*Solanum gilo*), with the aid of ImageJ[®] software,
261 allowed the measurement of free internal areas at different stages of maturation, as well as the
262 determination of the relationship between those and germination. Seeds of fruits harvested at 45 DAA
263 and seven days at rest showed a smaller free internal area and a formation of 100% of normal
264 seedlings.

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270 **4. CONCLUSIONS**

271 The analysis of radiographic images of jiló seeds (*Solanum gilo*), with the aid of ImageJ®
272 software, allow the measurement of free internal areas at different stages of maturation, as well as the
273 determination of the relationship between those and germination. The seeds of fruits harvested at 45
274 days after anthesis and seven days at rest (45DAAr) present a smaller free internal area and a
275 formation of 100% of normal seedlings, being this the ideal harvesting point.

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277 **CONFLICT OF INTEREST**

278 The authors declare no conflict of interest.

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